Chromosomal Staining Comparison of Plant Cells with Black Glutinous Rice (*Oryza sativa* L.) and Lac (*Laccifer lacca* Kerr)

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Summary The study on chromosomal staining comparison of plant cells with natural dyes was carried out to compromise the use of expensive dyes. Dyes from black glutinous rice (*Oryza sativa* L.) and Lac (*Laccifer lacca* Kerr) were extracted using acetic acid, ethanol, butanol and hexane with the concentration levels of 30%, 45% and 60%, respectively. The pH was then adjusted from 1 to 7, the natural extracted dyes were used to stain the chromosomes of spider lily (*Hymenocallis littoralis* Salisb.) root cells, which were ongoing mitotic cell division, using the squash technique. The results showed that the natural extract dyes were capable of chromosome staining and cell division observing. Natural dyes which showed well-stained chromosome included 45% acetic acid-extracted black glutinous rice dye (pH 1–3), 45% butanol-extracted black glutinous rice dye (pH 1–3) and 60% ethanol-extracted Lac dye (pH 1–3). We also concluded that all other extracts have no significant quality as chromosomal staining indication.

Key words Natural dye, Chromosome staining, Black glutinous rice (*Oryza sativa* L.), Lac (*Laccifer lacca* Kerr), Spider lily (*Hymenocallis littoralis* Salisb.).

Natural dye is well known as the color obtained from several natural resources such as that parts, including bark, leaves, fruits, flowers and roots, and also obtained from animals and minerals. The advantages of natural dye are that it is not harmful, has no toxicity and gives no flashy color. Natural dye is generally used as food coloration and also used as an accessory called dye, such as silk staining with curcuma (*Curcuma longa* L.) extract (Rochananark 1996, Sartpan 1993). The applications of dye from natural resources as a color accessory should be widely promoted because of its properties, such as low cost and low harmful effect in comparison with synthetic dye.

There are 3 types of natural dye

1) Inorganic mineral dye present as follows: in metallic oxides which leads to permanent staining, for example the yellow from Lead (II) chromate (PbCrO_4), the iron-rust color (khaki) from a combination of Ferric oxide (Fe_2O_3), Ferrous oxide (FeO) and Chromium (III) oxide (Cr_2O_3).

2) The dye obtained from animals, such as the orange from cochineal (*Coccus cacti*) and the purple-red from Lac (*Laccifer lacca* Kerr).

3) The dye from parts of plant including root, stem, bark, wood, leaves, flowers, fruit and seeds, for example the yellow from jackfruit wood (*Artocarpus heterophyllus* Lam.) and curcuma rhizome, the red from the Sappan tree stem (*Caesalpinia sappan* L.) and Indian mulberry root

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(*Morinda citrifolia* L.), the blue from true indigo leaves (*Indigofera tinctoria* L.) and Woad leaves (*Strobilanthes cusia* Kuntze) and the green from Bengal almond leaves (*Terminalia catappa* L.), Myrabolan bark (*Terminalia chebula* Retz) and Damocles bark (*Oroxylum indicum* Kurg) (Haewhareon 1999).

The reports of Kaveewong and Chaisomparn (2003), Tan (2003) and Wonkaonoi (2004) demonstrated that black glutinous rice (*Oryza sativa* L.) and Lac were useful in plant chromosome staining. In our study, we aim to determine the types of solvents, concentrations and pH levels which are optimum for the chromosome staining of spider lily (*Hymenocallis littoralis* Salisb., $2n=46$). Therefore, the present study will use natural dye as a substitute for synthetic dye in plant chromosome staining.

Materials and methods

Preparation of Natural Dye

Black glutinous rice and Lac were extracted by several solvents; 30% ethanol, 45% ethanol, 60% ethanol, 30% butanol, 45% butanol, 60% butanol, 100% hexane and 45% acetic acid. One hundred grams of natural dye were dissolved in 100 milliliters of each solvent for 24 h. Each solution was filtrated then adjusted for 7 levels of pH from 1–7.

Preparation of spider lily root chromosome

The 11 d growth of spider lily roots were shaped to about 1 centimeter. All were pretreated by being soaked in 0.2% colchicine for 16–18 h. Then they were fixed in absolute ethanol: glacial acetic acid in proportion of 3 : 1 for 12–24 h. After that, they were rinsed with 70% ethanol 2–3 times and kept in 70% ethanol.

Detection of chromosome staining

Each natural dye sample obtained from several treatments was used for spider lily root chromosome staining. All metaphase chromosomes were accomplished by the squash technique (Chaiyasut 1989, Campiranon 2003). All were detected under a light compound microscope and were photographed. Then, chromosome staining between natural dyes and synthetic dyes were compared (Aceto-orcein and Giemsa's).

Results and discussions

In our scheme, 4 organic solvents (acetic acid, butanol, ethanol and hexane) were used to extract black glutinous rice and Lac; we found that hexane is not capable of extracting the natural dye from black glutinous rice and Lac. Although ethanol can extract natural dye, it evaporated quite fast. Therefore, we concluded that acetic acid and butanol are the optimum solvents for natural dyes extraction. It is in agreement with the reports of Kaveewong and Chaisomparn (2003), Tan (2003) and Wonkaonoi (2004) in which acetic acid was used as solvent.

Several concentrations of organic solvents (45% acetic acid, 30% butanol, 45% butanol, 60% butanol, 30% ethanol, 45% ethanol and 60% ethanol) are capable of extracting natural dye from black glutinous rice and Lac. The results demonstrated that 45% acetic acid is good for staining the chromosome of spider lily, with obvious mitotic cell division (interphase, prophase, metaphase, anaphase and telophase). It is consistent with the report of Kaveewong and Chaisomparn (2003) that showed the clear observation of the multiplier onion root (*Allium cepa* var. *aggregatum*) metaphase chromosome, stained with black glutinous rice which was extracted with 45% acetic acid. Natural dyes from black glutinous rice and Lac, that were extracted with other solvents (30% butanol, 45% butanol, 60% butanol, 30% ethanol, 45% ethanol and 60% ethanol), can stain both

Fig. 1. Type comparative of solvents and concentrations potential on spider lily (*Hymenocallis littoralis*) chromosome staining extracted from black glutinous rice (*Oryza sativa*) and Lac (*Laccifer lacca*); A to G=black glutinous rice, H to N=Lac, A and H=45% acetic acid, B and I=30% Butanol, C and J=45% butanol, D and K=60% butanol, E and L=30% ethanol, F and M=45% ethanol, G and N=60% ethanol, scale bars 10 μ m.

Fig. 2. Comparison of pH levels potential on spider lily (*Hymenocallis littoralis*) chromosome staining extracted from black glutinous rice with 45% acetic acid at pH 1.18 (A), 2.18 (B), 3.18 (C), 4.18 (D), 5.18 (E), 6.18 (F), 7.18 (G); 45% butanol at pH 1.64 (A), 2.64 (B), 3.64 (C), 4.64 (D), 5.64 (E), 6.64 (F), 7.64 (G) and 30% ethanol at pH 1.40 (A), 2.40 (B), 3.40 (C), 4.40 (D), 5.40 (E), 6.40 (F), 7.40 (G), scale bars 10 μ m.

Fig. 3. Comparison of pH level potential on spider lily (*Hymenocallis littoralis*) chromosome staining extracted from Lac with 45% acetic acid at pH 1.62 (A), 2.62 (B), 3.62 (C), 4.62 (D), 5.62 (E), 6.62 (F), 7.62 (G); 60% butanol at pH 1.54 (A), 2.54 (B), 3.54 (C), 4.54 (D), 5.54 (E), 6.54 (F), 7.54 (G) and 60% ethanol at pH 1.35 (A), 2.35 (B), 3.35 (C), 4.35 (D), 5.35 (E), 6.35 (F), 7.35 (G), scale bars 10 μ m.

Fig. 4. The well-stained chromosome from 3 natural dyes extracted with 1) black glutinous rice in 45% acetic acid at pH 1–3; 2) black glutinous rice in 45% butanol at pH 1–3 and 3) Lac in 60% ethanol at pH 1–3. Mitotic cell division on interphase (A), prophase (B), metaphase (C), anaphase (D) and telophase (E), scale bars 10 μ m.

Fig. 5. Comparison of spider lily (*Hymenocallis littoralis*) chromosome staining between natural dyes and synthetic dyes; black glutinous rice in 45% acetic acid at pH 1.18 (A); black glutinous rice in 30% butanol at pH 1.69 (B); black glutinous rice in 45% butanol at pH 1.64 (C); Lac in 60% ethanol at pH 2.35 (D); acetone-orcein 20% (E) and Gimsa's 20%, scale bars 10 μ m.

the nucleus and cytoplasm, making it impossible to define the mitotic cell division (Fig. 1). We suggest that the type of organic solvent is influence on plant chromosome staining.

Natural dye from black glutinous rice and Lac as described above with pH levels 1–7 showed that natural dye with 45% acetic acid can stain plant chromosomes and define mitotic cell division well at pH 1–3 but pH 4–7 cannot define between nucleus and cytoplasm staining. Natural dye from other solvents (30% butanol, 45% butanol, 60% butanol, 30% ethanol, 45% ethanol and 60% ethanol) showed well-defined results, similar to 45% acetic acid (Figs. 2, 3). Moreover, we found that natural dye from black glutinous rice and Lac at pH 6–7 can result in obvious nucleolus staining (Fig. 6).

We suggest that the pH level of natural dye from black glutinous rice and Lac is an important factor in plant chromosome staining because it is in agreement with the report of Sukhumanpan (2002) that stained mulberry paper with natural dye from Roselle petal (*Hibiscus sabdariffa* L.) and

Fig. 6. Obviously plant nucleolus stained with natural dyes (arrow heads) A to D=black glutinous rice, E to F=lac, A=45% butanol at pH 6–7, B=60% butanol at pH 6–7, C=30% ethanol at pH 6–7, D=45% ethanol at pH 6–7, E=45% butanol at pH 6–7 and F=45% ethanol at pH 6–7, scale bars 10 μ m.

that of Wonkaonoi (2004) which showed that several natural dyes can stain crinum lily (*Crinum asiaticum* L.) chromosomes. The two reports above confirmed that differing pH levels have an influence on staining.

Natural dyes from black glutinous rice and Lac in several solvents, concentrations and pH levels have nearly as much potential as synthetic dyes (aceto-orcein and Giemsa's) for plant chromosome staining. Three optimum formulas are as follows;

1. Black glutinous rice with 45% acetic acid at pH 1–3
2. Black glutinous rice with 45% butanol at pH 1–3 and
3. Lac with 60% ethanol at pH 1–3 (Figs. 4, 5).

Furthermore, we found that the spider lily chromosome has $2n=46$ according to the staining of natural dyes; this is also consistent with the report of Lavinsh (2001).

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